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ON

EXPERIMENTAL STUDIES FOR THE DETECTION OF PROTEIN IN TRACE AMOUNTS (J-BANDS)

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The influence of pH on the intensity of the J-band formed in the presence of proteins was determined by mixing gelatin and 0.001M cacodylic acid or tris buffers with 3,3' diethyl -9 methyl 4,5,4',5' dibenzothiacarbocyanine bromide (I). It was found that, over the pH range 5.2 to 8.8, the intensity of the J-band was not appreciably altered.

The intensity of J-bands formed with E. coli suspensions and gelatine solutions in cacodylic acid buffer (pH 7.0) was investigated as a function of protein concentration. In each case, as little as 0.00005 percent protein gave an easily detectable J-band and increasing the protein concentrations, up to at least 0.1 percent, gave rise to progressively more intense bands.

The interaction of the dye with pyrimidine and purine bases, nucleosides and nucleotides was investigated at pH 7.0. Only the nucleotides altered the absorption spectrum of the dye and for each nucleotide tested an intense J-band was formed. Since the only difference between the nucleosides and the nucleotides is the presence of a phosphate group in the latter, it appeared that this group was responsible for the formation of the J-bands. At pH 7 the phosphate group is negatively charged. To determine if the phosphate group per se or simply the negative charge was responsible for this behavior, the reaction of cytidine $1/2H_2SO_4$ with the dye was investigated and it was found that this substance also caused the formation of J-bands. Apparently, strongly negatively charged sites are potent factors in the formation of J-bands. Therefore, the influence of inorganic anions on the formation of J-bands was ascertained. It was found that in the presence of inorganic salts the dye formed J-bands, but the salt concentrations required were very much greater than for proteins and several times greater than for the nucleotides. Chromate, sulfates, phosphates, chlorides, bromides, carbonates and nitrates were

effective with increasing potency in the order given, the divalent anions had the greatest effect and the cation species had little influence on the reaction of the dye. It was found that in the presence of 10 percent ethanol the salt concentration required to cause the formation of a J-band was increased tenfold, without altering the sensitivity to gelatin.

The interaction of the dye with forty amino acids or amino acid derivatives was determined in cacodylic acid buffer at pH 7.0. In no case was the absorption spectrum of the dye altered by the presence of these compounds.

The behavior of 3,3'-dimethyl 4,5,4',5' dibenzo-9 ethyl-thiacarbocyanine chloride with gelatin, sulfate, and nucleotide solutions was investigated. It was found that this dye forms an intense J-band at 650 mμ in the presence of these substances and that its sensitivity to sulfate ions was considerably greater than that of dye I.